

Investigations of the Toxic & Teratogenic Effects of GRAS Substances to the Developing
Chicken Embryo-Report of the investigation of Sodium Benzoate in the developing
chicken embryo

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SUBJECT: Investigations of the Toxic and Teratogenic Effects of GRAS
Substances to the Developing Chicken Embryo

Attached is the report of the investigation of SODIUM
BENZOATE in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of
GRAS Substances to the Developing Chicken Embryo:

SODIUM BENZOATE

PROTOCOL:

Sodium benzoate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in 5% of glucose with water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2, 3). For the purpose of determining the possible effect of the glucose solution as the solvent, double distilled water was substituted for the 5% water solution of glucose in a test in which a smaller number of eggs were treated via the air cell.

Groups of ten or more eggs were treated under these four conditions at several dose levels until a suitable total number of eggs per level was reached for all levels allowing some to hatch. Groups of adequate size were treated solely with the solvent at corresponding volumes. Untreated controls were also included in each experiment.

After treatment, all the eggs were candled daily and the non-viable embryos were removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. Along with these, histological examinations of major organs (liver, heart, kidney, lung, brain, intestine, gonad, and some endocrine organs) were carried out by taking samples from a representative number of animals from each experimental group.

RESULTS:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of the test.

Columns 1 and 2 give the dose administered in milligrams per egg and milligrams per kilogram egg weight, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated. This number has not been corrected for the sterile eggs or the eggs discarded due to accidents, thus providing a slightly higher mortality rate and a lower abnormality rate than was the actual case.

Column 4 is the percent mortality, i. e., the total number of non-viable eggs divided by the total number of treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total number of eggs treated. This includes all the abnormalities observed and also the toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia, and neural disorders including ataxia.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as a percentage of the total number of eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

The comparable data for the solvent treated eggs as well as the untreated controls are included in columns 3 through 6.

The mortality data in column 4 has been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data in columns 4, 5 and 6 has been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

DISCUSSION:

In this experiment, in all groups of eggs including those of the controls, the mortality rate was higher than usual because of unexpected mechanical underfunction of the incubation apparatus. Nevertheless, sodium benzoate was found to be not embryotoxic when administered to the chicken embryos under all of the conditions of the test. At no dose level was the toxicity significantly ($P=0.05$) greater than it was for the solvent-treated eggs. Probit analysis resulted in an LC_{50} of 0.463 mg/egg (yolk at 0 hours, Table 3). The air cell treatment at 0 and 96 hours and the yolk treatment at 96 hours each result in a line whose slope was not significantly different from zero (Tables 1, 2 and 3).

Abnormal birds were seen under all of the conditions of the test, although the incidence of birds having a structural abnormality of the head, limbs, viscera, or skeleton was not significantly different from that of the solvent background ($P=0.05$). Of the 152 untreated control embryos, only one was abnormal with curled toes which is a very frequently seen minor anomaly.

AIR CELL AT 0 HOURS: At 10 mg/egg there were three abnormal birds, all of which had curled toes. At 5.0 mg/egg two birds had curled toes and one bird had hip contracture. At 1.0 mg/egg there were three birds whose abnormalities included phocomelia, hip contracture, and curled toes. The solvent-treated eggs had no abnormal birds.

AIR CELL AT 96 HOURS: At 10 mg/egg two birds had curled toes. At 5.0 mg/egg there were six abnormal birds; four with curled toes, one with hip contracture, one with celosomia. No abnormality was found with solvent-treated eggs.

YOLK AT 0 HOURS: The 5.0 mg/egg was the only level that exhibited an abnormality, one bird with curled toes.

YOLK AT 96 HOURS: At 10 mg/egg four abnormal birds were seen; one with celosomia and three with curled toes. At 5.0 mg/egg four birds had curled toes. At 1.0 mg/egg four birds had curled toes and another one had hip contracture, while the solvent-treated control had no abnormality.

The result from the air cell experiment, in which in a limited number of eggs double distilled water replaced the 5% water solution of glucose as the solvent, was compared with that of the glucose solution group. From these it was concluded that there was virtually no difference in the effect between these two solvents on either the toxicity or the incidence and variety of abnormality found.

Microscopical examination of the paraffin embedded and H&E stained sections revealed no consistent histological changes in any of the organs observed.

Judging from all of these test results, it is concluded that sodium benzoate is neither toxic nor teratogenic to the chicken embryo. Most of the abnormalities found in this test were also observed in the solvent-treated or untreated controls.

1. Sodium benzoate, FDA 71-37
2. McLaughlin, J., Jr., Marliac, J.-P., Verrett, M. J., Mutchler, M. K. and Fitzhugh, O. G. Toxicol. Appl. Pharmacol. 5:760-770, 1963
3. Verrett, M. J., Marliac, J.-P. and McLaughlin, J., Jr. JAOAC 47: 1002-1006, 1964
4. Finney, D. J. Probit Analysis, 2nd ed., Cambridge Press, Cambridge, Appendix I, 1964

Table 1

Sodium Benzoate

Air Cell at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.0	200	153	77.12	1.96	1.96
5.0	100	155	84.51	1.93	1.93
1.0	20	151	64.23	1.98	1.98
5% Glucose in water		157	74.52	0	0
Control		152	73.02	0.65	0.65

Slope is negative

* Significantly different from solvent $P \leq 0.05$

Table 2

Sodium Benzoate

Air Cell at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.0	200	138	72.46	1.44	1.44
5.0	100	123	49.59	4.87	4.87
1.0	20	133	61.65	2.25	2.25
5% Glucose in water		134	50.74	0.74	0.74
Control		152	73.02	0.65	0.65

Slope is negative

* Significantly different from solvent $P \leq 0.05$

Table 3

Sodium Benzoate

Yolk at 0 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.0	200	176	100	0	0
5.0	100	178	97.19	0.56	0.56
1.0	20	179	94.97	0	0
5% Glucose in water		178	92.13	0	0
Control		152	73.02	0.65	0.65

LC₃₀ 0.178 mg/egg (3.564 mg/kg)

LC₅₀ 0.463 mg/egg (9.271 mg/kg)

LC₉₀ 4.794 mg/egg (95.899 mg/kg)

* Significantly different from solvent $P \leq 0.05$

Table 4

Sodium Benzoate

Yolk at 96 Hours

Dose		Number of eggs	Percent Mortality	Percent Abnormal	
mg/egg	mg/kg			Total	Structural
10.0	200	236	85.16	1.69	1.69
5.0	100	237	81.43	1.68	1.68
1.0	20	242	80.99	2.06	2.06
5% Glucose in water		167	80.83	0	0
Control		152	73.02	0.65	0.65

Slope is negative

*Significantly different from solvent $P \leq 0.05$